IN VITRO SHOOT MULTIPLICATION AND PLANTS REGENERATION FROM SINGLE SHOOT OF CHRISTMAS CACTUS (*SCHLUMBERGERA RUSSELLIANA*)

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ABSTRACT

This study was conducted at Laboratory of plant tissue culture - Department of Horticulture and Landscape Design, College of Agriculture - Basra University, During the year of 2016/ 2017. The aim of this study was to test the different concentrations of Cytokinin and Auxin on shoot multiplication and rooting of Christmas cactus (*Schlumergera russelliana*) plant. the results of first experiment showed that single shoot (cladode) culturing on MS medium supplemented with 0.5 and 1.0 mg. L^{-1} of Thidiazuron combined with 0.2 mg. L^{-1} NAA gave a high numbers of shoots, shoot length and width (6.33, 6.00 shoot/ explants, 2.10, 1.83 cm and 1.73,1.70 cm) respectively as compared with other treatments (0.0, 2.0 and 3.0 mg. L^{-1} TDZ). Results also revealed that all treatments showed appearance of thin roots. Second experiment showed that 8.0 mg. L^{-1} IAA combined with 0.2 mg. L^{-1} BA gave a longest roots and plants (14.00 and 9.23 cm) as compared with other treatments (0.0, 2.0, 4.0 and 6.0 mg. L^{-1} IAA. All treatments of IAA gave 100% of root formation. The plants produced by micropropagation have been acclimatized at rate of 80%.

Key word : *In vitro*, Thidiazuron, Indol acetic acid, Single cladode, Christmas cactus

1. INTRODUCTION

Christmas cactus belongs to *Schlumergera russelliana* in Cactaceae family, Buckley groups. This genus (*Schlumergera*) contains the popular house plants known by the variety of names including Christmas cactus, thanks giving cactus, Crab cactus and Holiday cactus which are *Schlumergera* cultivars, [1]. It's endemic to a small area of the coastal mountains of the south eastern Brazil where is natural habitat is moist forest, It grows on tree as epiphyte, Christmas cacti have fleshy green stems which act as photosynthic organs, The stem (Cladodes) divided into flattened leaf- like segments are about 1- 3.8 cm long by 0.8 – 2.0 cm on wide (Fig,1) Cladodes of Christmas cactus with rounded more symmetrical teeth [2].

Genus *Schlumbergera* has a succulent plants and can store a reasonable quantity of water, it is not a true cactus, This plant commonly propagated by taking stem or shoot cutting of the stem tips which called areoles, This conventional may is aslow process of propagation and they are subjected to many diseases and environmental hazards, that cause gradual degeneration of cultivar [3].

In recent of years, cell and tissue culture techniques have been successfully used for mass production of cacti, and this techniques may facilitated their propagation over a shorter time period than conventional techniques used for commercial purposes [3].

Thidiazuron (TDZ) is the Cytokinin which have a major role on plant development, such as the regulation of shoot development and multiplication and improvement of cell division and expansion [5].

Rahimi *et al.* [6] produced the highest percentage response of *Fritillaria imperialis* L. plants to shoot multiplication when their explants were cultured on MS medium supplemented with 0.5 mg. L^{-1} TDZ .However Al-Taha and Al-Mazine [7] obtained largest number of shoots when shoot tips of *Gardenia jasminoids* cultured on MS medium contained 0.5and 1.0 mg. L^{-1} TDZ.

For rooting shoots, Duhoky and Rasheed [8] reported that, the average number and length of roots were obtained when shoots of *Gardenia jasminoids* were cultured on MS medium supplied with 8.0 mg. L^{-1} IAA.

A semilare results were found when *Gardenia jasminoids* cv "Radicans" shoots were cultured on MS medium supplied with 8 mg. L^{-1} NAA [9].

The objects of this study were to develop methods for the mass scale production of healthy and premium quality planting materials of Christmas cactus and achieve to study the behavior of conceptive *In Vitro* propagation stages of Christmas cactus, shootlet proliferation stage by using different concentrations of TDZ and IAA treatments for rooting and acclimatization stage.

Fig. 1. Chrictmas Cactus plant produce from tissue culture

2. MATERIALS AND METHODS

This study was carried out at tissue culture laboratory – College of Agriculture – Basra University –Iraq. to study the effect of :

2.1 Effect of Different concentrations of Thidiazuron (TDZ) on shoot multiplication:

Multiple shoots were produced from aseptic culture of Christmas cactus (fig, 2 A, B, C). In this experiment, a single shoot (cladode) was cultured vertically in full strength of MS medium [10] supplemented with Organic compounds (table, 1) This medium was supplemented with different concentrations of TDZ (0.0 , 0.5, 1.0 , 1.5 and 3.0 mg. L^{-1}) and 0.2 mg. L^{-1} NAA for each treatments. The cultures were grown in growth room at 25 ± 2 C (16 h light and 8 h Dark), Every treatment was replicated 10 times the data was recorded after 12 weeks of culture.

The Studied characteristics were :

1- Number of shoot (cladode)/ explant
2- Length and width of shoot (cm).
3- Callus formation.

2.2 Effect of different concentrations of IAA (Indol acetic acid) on rooting of shoots:-

The produced shoots by multiplication (each plantlet had two cladode) were cultured on the same medium of multiplication except that TDZ was replaced by different concentrations of IAA (0.0, 2.0, 4.0, 6.0 and 8.0 mg. L^{-1}) and 0.2 mg/l BA for each treatment.

Table 1. The chemical composition additives to MS medium

Seq	Chemical material	Quantily mg . L^{-1}
1	Sucrose	30000
2	Adenine sulphate	40
3	Polyvinyl pyrolodine	500
4	Glycine , Thiamins, pyridoxine	1
5	Agar	5000
6	Citric acid + Ascarbic acid	150 + 100
MS	Murashige and skoog , 1962	4.33

Every treatment in this experiment was replicated 10 times. The cultures were grown in the same conditions of the multiplication experiment, Data was recorded after 12 weeks recorded which included the following:-

1- The percentage of response for shoot rooting (%) .
2- Root length (cm)
3- Length of plantlets (cm)

2.3 Acclimatization of Christmas cactus:

The Christmas cactus plants lets produced from rooted shoos were removed from culture vessels and washed with sterile water to remove the traces of Agar, The plants were placed in glass tubes containing distilled water (Fig, 2 G) for 8 – 10 was changed every two days, then planted in plastic pots 5 cm in diameter containing autoclaved soil mix (Petmoss and ground sand (1:2), covered with glasses during 10-15 days to encourage acclimatization then the glasses cover was removed gradually to harden them higher temperatures and light intensity with lower air moisture and kept in the culture room under controlled conditions (Temperature 25 ± 2 C, 24 hours photoperiod with1500 Lux light intensity), After 12 weeks plants were transferred into large plastic pots 15 cm in diameter containing the same soil (Fig,2 G, H, I, J, K).

The percentage of survival was recorded after transfer by using the following equation

$$\% \text{ of frequency} = \frac{\text{Number of explants showing response}}{\text{Total Number of inoculated explants}} \times 100$$

3. RESULTS AND DISCUSSIONS

Effect of different concentrations of TDZ on shoot multiplication:

Results from (table, 2) shows the positive response to application of cytokinin TDZ in culture medium supplemented with(0.0, 0.5, 1.0, 2.0 and 3.0 mg. L^{-1}) TDZ in combination with 0.2 mg. L^{-1} of Auxine NAA after 12 week of culture (Fig, 2 D). The treatment with 0.5, 1.0 mg. L^{-1} TDZ gave the best number of shoots averaging (6.33, 6.00) shoot / explant, Length of shoot (21.0, 1.83cm) and width of shoot (1.73, 1.70 cm) respectively, this treatments were significant with other treatments (0.0, 2.0, 3.0 mg. L^{-1}) TDZ respectively.

This response is due to the fact that TDZ is stable and more active at lower concentration than the Adenine – type Cytokinin Mok *et al*. [11], However TDZ causes the breaking of the apical dominance, and thus increases multiplication areas and cell elongation through physiological changes same what elucidated that Guo *et al*. [12], More ever Hare *et al*. [13], reported that TDZ hinders the action of Cytokinin – Oxidase and this allows the proliferation of meristematic zones in explants

(Table, 2) also shows a decrease in shoots number and length with increasing concentration of the TDZ in medium from 0.5 to 3.0 mg. L^{-1} . which was reached to the low number (1.66 shoot/explants) and (1.30cm) length and width of shoot (1.26 cm) respectively.

The reason for the low numbers of shoots at high concentration of TDZ in medium is due the presence of high TDZ concentration in medium, these concentration of TDZ reduces the role of endogenous auxin in stimulating cell elongation [14].

These results are in a agreements 'with results of other studies in other plants as ceteus peruvianus [15] and [16] *Hippeastrum hybridum* and [6] *Fritillaria lmperialis* and [7] *Gardenia jasminoid* Ellis cv "Radicans".

The results also indicated (Fig, 2 D) that all the concentrations of TDZ and control were very good for formation of rootless from *in vitro* plantlets, this might is due to the cacti plants, In general is known to produce high levels of Auxins Hubstenberger *et al*. [17] and this was evident in ready rooting ability of initial cultures in the present study. Similar results were reported by Karimi *et al*. [18] in micropropagation of *Cereus peruvianus* , which revealed that 4.0 mg. L^{-1} of 2, 4-D + 2 mg . L^{-1} Kin and 4 mg. L^{-1} NAA + 2.0 mg. L^{-1} Kin were very good for formation of rootles.

Results also indicated (Fig, 2D) that callus formation was found on the edges and on the apical of Areoles which have ameristematic tissue, this was in agreements with Ruminska and Kulus [19], Moreover many cacti plants produce large amounts of Auxin under *in vitro* conditions that stimulating callus proliferation Clayton *et al*. [20] similar results were reported in other plants also used BAP, 2,4-D, NAA and TDZ for callus indication in *Cereus peruvianus* Karimi *et al*. [18] *Falcaria vulgaris* [21] and *Gardenia jasminoid* Ellis cv "Radicans" [7].

Table 2. Effect of different concentrations of TDZ on shoot (cladodes) multiplication

Concentration of TDZ mg . L-¹	Number Shoot Shoot / exblants	Length shoot Exblants / cm	Width shoot Exblants / cm
0.0	2.00	1.63	1.16
0.5	6.33	2.10	1.73
1.0	6.00	1.83	1.70
2.0	2.66	1.70	1.40
3.0	1.66	1.30	1.26
R.L.S.D 0.05	0.96	0.30	0.08

Effect of different concentrations IAA on shoot rooting :-

Results in(table, 3) shows that shoots from multiplication stages cultured on Ms medium supplemented with different concentrations of Auxine IAA (2.0, 4.0, 6.0 And 8.0 mg. L^{-1}) in combination with 0.2 mg. L^{-1} BA after 12 weeks of culture for Christmas cactus plant. All the concentrations of IAA gave the highest response percentages of rooting which reached 100%, the might due to that Cacti plants have high level of endogenous Auxin, rooting can occur spontaneously with of PGR (plant Growth regulators) *in vitro* conditions, eliminating the need for rooting medium and reducing cost and time when added PGR were added to culture medium {[20] and [17]}.

These finding are in agreement with those reported by {[22] and [23]} on *Cephalocereu*s *sinilis* and *Opentia ficus – indica* cacti plants respectively. The highest rates of roots density(++++) and the high average of, root length and plant length(14.00 cm) and length of (9.23 cm) respectively was obtained in MS medium containing 8.0 mg. L^{-1} IAA. This treatment was significant with other concentrations (0.0, 2.0, 4.0 and 6.0) mg. L^{-1} IAA which gave less response of root formation (Fig 2E,F).

The results proved that Auxins have a role in rooting presses since they promote the root initiation in the bases of cultured shoot. Root initial cells division depends on both Endogenous and exogenous auxins concentration. The physiological effects of auxins are represented in increasing of cell division or converting the material of differentiated cells in shoots bases into merastimatic cells (totipotent cells) so adventitious root meristem will be formed and its cells will be divided to produce adventitious roots [24] ; [25]}. Endogenous hromones might have arol in promoting shoots to root peak *et al*. [26] until the hormonal balance reached its optimal level to push the roots to grow and develop in presence of exogenous hormones, since increasing of auxin concentration promotes root formation on shoot bases [27].

Similar results were obtained by other researchers in their studies on *Melo cactus glaucescens. Cereus pruvianus, Cardemia jasminoides and Granderia jasminoides* cv"Radicans" plants {[28]; [18]; [8] and [9]}.

Table 3. Effect of different concentration of IAA shoot (cladodes) rooting

Concntrations of IAA mg . L-¹	Response of root formation %	root Length Shoot / cm	plant Length Shoot / cm	Root density
0.0	100	3.33	5.10	+
2.0	100	7.66	6.76	++
4.0	100	8.00	6.60	++
6.0	100	12.00	7.06	+++
8.0	100	14.00	9.23	++++
R.L.S.D 0.05		2.23	0.37	

Acclimatization:

Finally the healthy regenerated plantlets were acclimatized for four weeks under in itrocondition, The process of acclimatization continued for two months (Fig 2, G, H, I, J, K) and the rate of survival was 80%, because cacti like other plants, are greatly influenced by the controlled condition in tissue culture which may result in the death of plantlets Balen *et al*. [29]. Similar results were reported by other researchers in their studies on *Ceraos perunierous* plant Sayed *et al*. [30] and *pilosocereus robinuis* plant Quiala *et al*. [31] and *melococtus glaucas caus* [28].

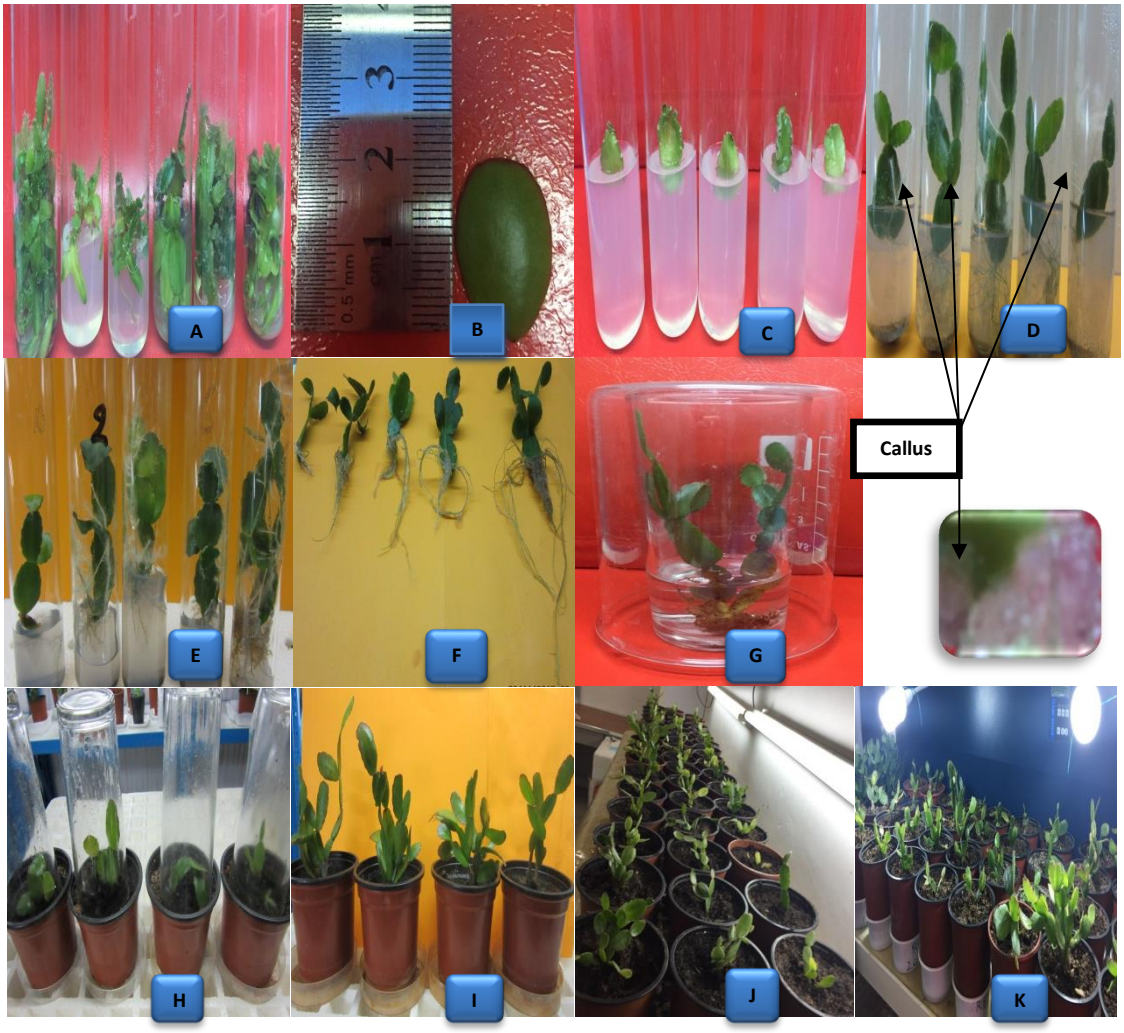

Fig. 2 Micropropgation of christmas cactus plant (*Schlumbergera russelliana*)(A) Aseptic culture on MS medim supplemented with 3.0 mg.L⁻¹ BA (B ,C) Single cladode about 1– 2 cm length and 0.8 – 1.0 cm width cutured vertically on MS medium(D) Shoot regeneration cultured on MS medim supplemented with different concentration of TDZ (0.0 ,0.5 ,1.0 ,2.0 ,3.0 mg.L⁻¹) +0.2 mg .L⁻¹ NAA Root formation and callus intiatiation from the apical of Arrole after 12 week (E ,F) Rooting shoot cultured an full MS medium supplemented with different concentration of IAA (0.0, 2.0, 4.0, 6.0, 8.0 mg. L⁻¹) + 0.2 mg . L⁻¹ BA after 12 weeks (G,H) Hardeing stage (I,J,K) Acclimatized plants after eight weeks.

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